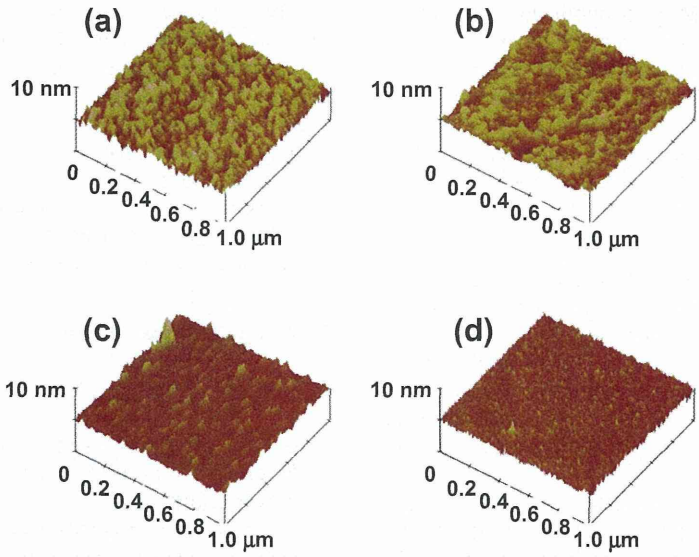

Fig. 3. Height images of (a) PMPC50 brush layer, (b) POEGMA50 brush layer, (c) PHEMA50 brush layer, and (d) BrC10DMCS-immobilized substrate, obtained by atomic force microscopy (AFM) under aqueous conditions.

The ellipsometric thickness values obtained for the grafted polymer layers under dry conditions were plotted against the absolute molecular weights of the polymer chains (Fig. 2). Thus, we demonstrated that the thickness of the grafted polymer layers could be linearly controlled in the range of 1–6 nm by controlling the molecular weight of the grafted polymer chains. We estimated the graft density of each polymer chain on the BrC10DMCS-immobilized substrates by using the slope of the linear graph to determine the relationship between thickness and molecular weight (Fig. 2). The graft densities of the polymer chains in the PMPC, POEGMA, and PHEMA brush layers were 0.26, 0.27, and 0.59 chains/nm^2, respectively. The graft densities of all polymer chains were greater than 0.10 chains/nm^2, which indicates the formation of dense polymer brush layers [27].

The surface topology of the polymer brush layers was examined using an AFM under aqueous conditions (Fig. 3). The surface of the BrC10DMCS-immobilized substrate was nearly flat, with an RMS value of exactly 0.40 nm (Fig. 3d). The surface roughness of each polymer brush layer increased compared with that of the BrC10DMCS-immobilized substrate (maximum RMS value: 0.8 nm) (Fig. 3a–c). These values were comparable to the previously reported RMS values in dry conditions [26], thereby indicating that the polymer brush layers prepared by the SI-ATRP method were considerably homogeneous and hardly changed in response to environmental conditions.

We measured the static water contact angles under dry conditions and static air bubble contact angles in aqueous conditions at the polymer brush layers, and plotted them against the ellipsometric thickness of the grafted polymer layers (Fig. 4). The static water contact angle in dry conditions for the silicon wafer was <10°, whereas that for the BrC10DMCS-immobilized silicon wafer was >80° (Fig. 4A). The static water contact angles for the polymer brush layers were lower than that of the BrC10TCS-immobilized substrate, even for thin grafted polymer layers. In all polymer brush layers, the static water contact angle under dry conditions decreased with increase in the thickness of the grafted polymer layers. The static water contact angles under dry conditions for PMPC brush layers decreased rapidly at a polymer chain thickness

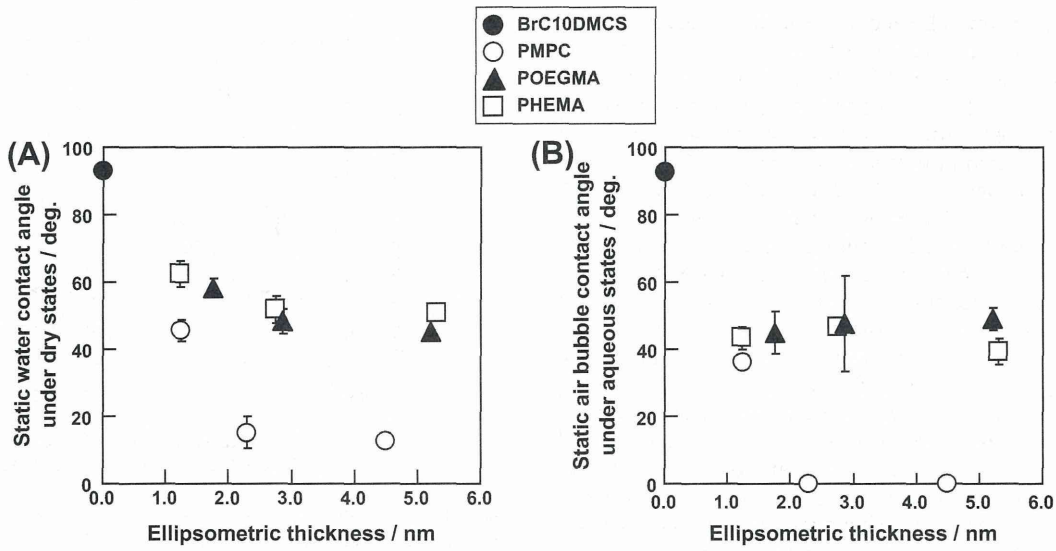

Fig. 4. (A) Static water contact angles under dry conditions and (B) static air bubble contact angles under wet conditions of the BrC10DMCS-immobilized substrate (closed circle), PMPC brush layers (open circles), POEGMA brush layers (closed triangles), and PHEMA brush layers (open squares).

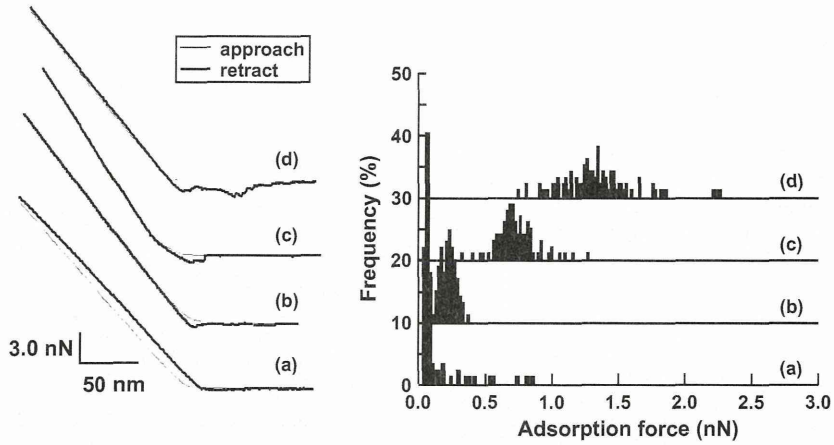

Fig. 5. (Left) Representative force-versus-distance curves between bovine serum albumin (BSA)-immobilized cantilever and polymer brush layers, and (Right) histograms of adhesion force of BSA against the polymer brush layers: (a) PMPC10 brush layer, (b) POEGMA10 brush layer, (c) PHEMA10 brush layer, and (d) BrC10DMCS-immobilized substrate.

of 2.0 nm, and then changed more slowly at approximately 10° at higher thickness values. On the other hand, the static water contact angles under dry conditions for the POEGMA and PHEMA brush layers were approximately 50° in the thick grafted polymer layers. Additionally, the static air bubble contact angles in aqueous conditions in all polymer brush layers did not change compared with the static water contact angles in dry conditions, indicating that the surface energy of the polymer brush layers remained constant in both conditions. Particularly, the high resistance to air bubbles in aqueous conditions of the thicker PMPC brush layers demonstrates that higher thickness confers high surface energy in aqueous media.

3.2. Adhesion force of proteins against polymer-grafted substrates

The extremely high repellency against protein adsorption of the hydrophilic polymer brush layers makes it difficult to discuss the slight differences in protein adsorption mass between the chemical structures of the polymer brush layers. Indeed, the amount of proteins adsorbed on PMPC, POEGMA, and PHEMA brush layers quantified by QCM measurement or radiolabeled proteins seems to be near the measurement limitation [9,10]. In this regard, the adhesion force of proteins against the surface of materials would be an important parameter that determines the protein adsorption behaviors at the interface.

Before the measurement of adhesion force of proteins against the polymer brush layers, the successful immobilization of proteins on the cantilever was confirmed by using QCM-D and XPS measurements (data not shown). From the QCM-D profiles, there was little difference in the BSA-immobilization process between with or without activation of COOH-SAM by WSC/NHS solution. The result indicated that the characteristics of the immobilized BSA on the cantilever surface are similar to that of the adsorbed BSA on the COOH-SAM [28,29].

Fig. 5 shows the representative *f–d* curves for the approach and retraction of a BSA-immobilized cantilever from the polymer brush layers or BrC10DMCS-immobilized substrates. In the case of the hydrophobic BrC10DMCS-immobilized substrate, the adhesion force of BSA was detected far from the outermost surface (up to 100 nm), and the top of the adhesion force was large (ca. 1.5 nN). On the other hand, the adhesion force of BSA was quite small and detected in a short range from the surface of the polymer brush layers. The high and long-ranged adhesion force against the BrC10DMCS-immobilized substrate indicates that the adsorbed BSA on the BrC10DMCS-immobilized substrate could hardly detach from the surface. Fig. 5 also shows the representative histograms of adhesion force of BSA against the thin polymer brush layers and BrC10DMCS-immobilized substrates from more than 100 *f–d* curves. The distribution of the adhesion force of BSA against the substrates could be fitted to Gaussian models and varied between different kinds of substrates. That is, the adhesion force against the BrC10DMCS-immobilized substrate had a broad distribution and large average value compared with that against the polymer brush layers with relatively thin layer thickness. Among the polymer brush layers, the adhesion force of BSA against the PHEMA10 brush layer was higher than against the other thin polymer brush layers. Fig. 6 shows the dependence on layer thickness of the polymer brush layers with different monomer units on the adhesion force of BSA against them. The adhesion force of BSA against the polymer brush layers decreased with the increase in layer thickness. This result was similar to previously reported results that the amount of the adsorbed proteins on the polymer brush surface depended on the thickness of the layer [10]. Furthermore, there was little difference in the adhesion force of BSA against the thick polymer brush layers among the chemical structures of monomer unit compared with the thin polymer brush layers. These results demonstrate the particularity of the polymer brush layer compared with the conventional materials that the thickness of the polymer brush layer has a relatively larger influence on the adhesion force of proteins than the chemical structure of the polymer brush layers. The proteins immobilized on the cantilever would maintain

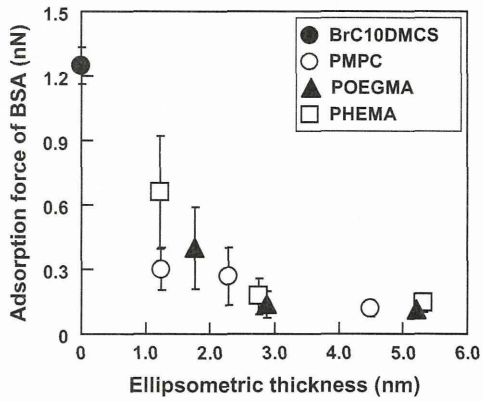

Fig. 6. Relationship between adhesion force of BSA and ellipsometric thickness at the PMPC brush layers (open circles), POEGMA brush layers (close triangles), PHEMA brush layers (open squares), and BrC10DMCS-immobilized substrate (closed circle).

their activity during the short measurement time of the *f–d* curves, while the conformation of proteins adsorbed on a surface would gradually change during the long measurement time for the protein adsorption mass. To better clarify the adhesion force of proteins against the polymer brush layers, the species and conformation of the proteins immobilized on the cantilever should be controlled. We are currently investigating the adhesion force of other or denatured proteins against polymer brush layers composed of hydrophilic monomer moieties, and hope to report these findings in the near future.

4. Conclusion

We synthesized three kinds of polymer brush layers, namely, PMPC, POEGMA, and PHEMA, on silicon wafers by using the SI-ATRP method. The graft density of polymer chains on the surface was high enough to form dense polymer brush layers. The surface roughness of the polymer brush layers under aqueous conditions analyzed using the AFM height images was quite low regardless of the structure of the monomer units. The wettability by water and resistance to air bubbles increased with the increase in thickness of the grafted polymer layers, and particularly, the PMPC brush layer had rather high surface energy. The adhesion force of BSA on the polymer brush layers decreased with increasing thickness of the grafted polymer layer, and finally reached an almost similar value among the different chemical structures. From these results, we conclude that the thickness of the polymer brush layer has a relatively larger influence on the adhesion force of proteins than the chemical structure of the polymer brush layers. We believe that the direct measurement of adhesion force of proteins against the nanostructure-controlled polymer brush layers would be a promising method to help elucidate the relationship among surface structure, surface properties, and protein adsorption behavior on the surface of biomaterials.

Acknowledgements

The authors thank Prof. Madoka Takai and Dr. Ryosuke Matsuno, The University of Tokyo, for helpful discussion on this research.

References

[1] H. Chen, L. Yuan, W. Song, Z. Wu, D. Li, Prog. Polym. Sci. 33 (2008) 1059–1087.
[2] E. Ostuni, R.G. Chapman, R.E. Holmlin, S. Takayama, G.M. Whitesides, A. Survey, Langmuir 17 (2001) 5605–5620.
[3] W. Norde, D. Gage, Langmuir 20 (2004) 4162–4167.
[4] G.L. Kenausis, J. Voros, D.L. Elbert, N. Huang, R. Hofer, L. Ruiz-Taylor, M. Textor, J.A. Hubbell, N.D. Spencer, J. Phys. Chem. B 104 (2000) 3298–3309.
[5] K. Ishihara, T. Ueda, N. Nakabayashi, Polym. J. 22 (1990) 355–360.
[6] K. Ishihara, H. Nomura, T. Mihara, K. Kurita, Y. Iwasaki, N. Nakabayashi, J. Biomed. Mater. Res. 39 (1998) 323–330.
[7] Y. Inoue, J. Watanabe, K. Ishihara, J. Colloid Interface Sci. 274 (2004) 465–471.
[8] Y. Goto, R. Matsuno, T. Konno, M. Takai, K. Ishihara, Biomacromolecules 9 (2008) 828–833.
[9] C. Yoshikawa, A. Goto, Y. Tsujii, T. Fukuda, T. Kimura, K. Yamamoto, A. Kishida, Macromolecules 39 (2006) 2284–2290.
[10] W. Feng, S. Zhu, K. Ishihara, J.L. Brash, Biointerphases 1 (2006) 50–60.
[11] Z. Wu, H. Chen, X. Liu, Y. Zhang, D. Li, H. Huang, Langmuir 25 (2009) 2900–2906.
[12] C.R. Emmenegger, E. Brynda, T. Riedel, Z. Sedlakova, M. Houska, A.B. Alles, Langmuir 25 (2009) 6328–6333.
[13] R. Iwata, P. Suk-In, V.P. Hoven, A. Takahara, K. Akiyoshi, Y. Iwasaki, Biomacromolecules 5 (2004) 2308–2314.
[14] Y. Inoue, K. Ishihara, Reduction of protein adsorption on well-characterized polymer brush layers with varying chemical structures, Colloids Surf. B: Biointerfaces 81 (2010) 350–357.
[15] Z. Zhang, M. Zhang, S. Chen, T.A. Horbett, B.D. Ratner, S. Jiang, Biomaterials 29 (2008) 4285–4291.
[16] W. Yang, S. Chen, G. Cheng, H. Vaisocherová, H. Xue, W. Li, J. Zhang, S. Jiang, Langmuir 24 (2008) 9211–9214.
[17] W. Yang, H. Xue, W. Li, J. Zhang, S. Jiang, Langmuir 25 (2009) 11911–11916.
[18] Z. Zhang, H. Vaisocherová, G. Cheng, W. Yang, H. Xue, S. Jiang, Biomacromolecules 9 (2008) 2686–2692.
[19] A. Idiris, S. Kidoaki, K. Usui, T. Maki, H. Suzuki, M. Ito, M. Aoki, Y. Hayashizaki, T. Matsuda, Biomacromolecules 6 (2005) 2776–2784.
[20] N.W. Moore, D.J. Mulder, T.L. Kuhl, Langmuir 24 (2008) 1212–1218.
[21] S. Kidoaki, T. Matsuda, Langmuir 15 (1999) 7639–7646.
[22] S. Kidoaki, Y. Nakayama, T. Matsuda, Langmuir 17 (2001) 1080–1087.
[23] A. Ramakrishnan, R. Dhamodharan, J. Ruhe, Macromol. Rapid Commun. 23 (2002) 612–616.
[24] M. Husseman, E.E. Malmstrom, M. McNamara, M. Mate, D. Mecerreyes, D.G. Benoit, J.L. Hedrick, P. Mansky, E. Huang, T.P. Russell, C.J. Hawker, Macromolecules 32 (1999) 1424–1431.
[25] K. Yamamoto, Y. Miwa, H. Tanaka, M. Sakaguchi, S. Shimada, J. Polym. Sci. A: Polym. Chem. 40 (2002) 3350–3359.
[26] W. Feng, J. Brash, S. Zhu, J. Polym. Sci. Part A: Polym. Chem 42 (2004) 2931–2942.
[27] Y. Tsujii, K. Ohno, S. Yamamoto, A. Goto, T. Fukuda, Adv. Polym. Sci. 197 (2006) 1–45.
[28] C.D. Tidwell, S.I. Ertel, B.D. Ratner, B.J. Tarasevich, S. Atre, D.L. Allara, Langmuir 13 (1997) 3404–3413.
[29] B. Sivaraman, K.P. Fears, R.A. Latour, Langmuir 25 (2009) 3050–3056.

Nano Today (2011) 6, 61—74

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REVIEW

Integrated functional nanocolloids covered with artificial cell membranes for biomedical applications

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Received 8 November 2010; received in revised form 14 December 2010; accepted 23 December 2010
Available online 17 January 2011

KEYWORDS
Nanocolloids;
Artificial cell membrane;
Surface modification;
2-Methacryloyloxyethyl phosphorylcholine (MPC) polymer;
Protein adsorption;
Cell adhesion

Summary The functionality of nanocolloids used in biomedical applications are subject to strong interference arising from significant interactions with biological components such as proteins and cells. Among the known examples of surface treatment of nanocolloids, the construction of an artificial cell membrane structure based on phospholipid polymers has proven effective in preventing the occurrence of biological reactions at the surface. Furthermore, certain bioactive molecules can be immobilized on the surface of the phospholipid polymer platform to generate bioaffinity for other biomolecules. This review describes preparation and characterization of integrated functional nanocolloids covered by artificial cell membrane structures and their performance in biomedical applications.

Introduction

Nanocolloids may be defined as nanometer-scale particles dispersed in a medium [1]. They are mainly composed of metals and metal oxides, polymers, semiconductors, and carbon nanotubes, and display exceptional physical and chemical characteristics relative to bulk materials. Nanocolloids can be dispersed into solutions to form suspensions since the interaction of the colloid surface with the solvent is strong enough to overcome density differences. The efficiency of catalysts that employ nanocolloids tends to be high due to the increased surface-to-volume ratios. At the nanometer-scale, size-dependent properties are often observed. For example, quantum confinement in semiconductor particles, surface plasmon resonance in certain metal particles and superparamagnetism in magnetic materials. Moreover, unique structures with various functions can be fabricated by assembly of nanocolloids [1,2]. The nanometer-scale sizes of these structures make them suitable for analysis of microscopic regions, such as targeted intracellular regions. Such nanocolloids are widely used in applications such as development of biomolecular-supported materials, specific stains, and thermally, magnetically, and optically responsive materials for

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doi:10.1016/j.nantod.2010.12.009

analyses of microscopic regions. In addition, nanocolloids are actively used in pharmaceutical and biomedical applications such as carriers for drugs and biomolecules and as imaging reagents.

However, when the nanocolloids are applied to biological systems, they encounter problems due to their specific structures. Since nanocolloids possess high energy surfaces, uncontrolled aggregation of colloid particles due to attractive van der Waals forces or electrostatic interactions gives rise to microstructural inhomogeneities in the medium. Protein becomes randomly adsorbed to the nanocolloid surface and reduces the function of the nanocolloids according to the size effect. The nanocolloids become adsorbed onto the cell membrane and enter the cell by endocytosis in the cell culture medium. The nanocolloids occasionally induce unexpected cellular responses. Regardless of the application of nanocolloids under biological conditions, the functionalities of nanocolloids may be disrupted by these biological responses, which are induced by nonspecific protein adsorption. To avoid this problem, the surfaces of nanocolloids are engineered to be biocompatible and bioinert [3–7].

There are many methodologies, which may be used to modify the surface of nanocolloids to obtain bioinert surfaces. Examples include the coupling of protein layers or self-assembling peptides to the surface, or coupling of water-soluble polymers to the surface. A rational strategy involves the use of bio-derived molecules. An adsorbed layer of bioinert protein, such as bovine serum albumin (BSA) or casein provides protein adsorption resistance [8]. These proteins are amphiphilic and have been used in the enzyme-linked immunosorbent assay (ELISA) as a blocking agent. Dextran, a natural polysaccharide, is also used as a surface modification to prevent bio-fouling [9]. Water-soluble synthetic polymers, such as poly(ethylene oxide) and polyacrylamide derivatives can be grafted or coated onto nanocolloids [10,11]. The water-soluble polymer chains expand into the aqueous medium and generate a highly mobile hydrated layer that surrounds the nanocolloids. Thus, the steric hindrance due to polymer chains prevents aggregation of the nanocolloids.

Other possibilities for generating surface modifications have been researched using phospholipid derivatives. Phosphatidylcholines are natural phospholipids that form self-assembly nanocolloids as a result of molecular interactions, which generate liposomes and lipid microspheres [12]. These phospholipid nanocolloids are used as pharmaceutical devices for carrying bioactive reagents in the bloodstream. Phosphatidylcholines have good biocompatibility, but their stability is not sufficient to enable long-term circulation in the bloodstream. However, the phospholipid assembly is one of the best candidate platforms for providing biocompatible surfaces. Moreover, when proteins and polysaccharides are immobilized on the platform, they adopt essentially the same structures that they have when they are attached to the cell membrane. Examples of monomers bearing phospholipid polar groups, notably the phosphorylcholine (PC) group, have reported [13–19]. These monomers can be polymerized and copolymerized with other vinyl compounds with various functional groups. Thus, the phospholipid polymers obtained show an interesting property in an aqueous medium.

Among these phospholipid polymers, 2-methacryloyloxyethyl phosphorylcholine (MPC) polymers are the most successful biomaterials for improving the biocompatibility of the surfaces of medical devices. The MPC polymers have PC groups in their side chains and have variable chemical structures as a result of the copolymerization process (Fig. 1) [19]. Over the past decade it has been established that the molecular weight, chain length, polydispersivity, conformation, and composition of the polymers can be controlled using living radical polymerization technique such as atom transfer radical polymerization (ATRP) [20], reversible addition-fragmentation transfer (RAFT) polymerization [21] and photoinduced iniferter polymerization [22]. These polymerization methods have been applied for obtaining the MPC polymers [23–27]. The MPC polymers have hydrophilicity and are electrically neutral due to their zwitterionic structures. They have low-friction and fluidity in aqueous media and have stable conformations in high salt solution and over a wide pH range. They are also biologically inert and have resistance to protein adsorption and cell adhesion. Furthermore, they do not cause tissue immunoreactions [28–34]. Thus, the modification of nanocolloids with the MPC polymers provides a bioinert platform similar to a cell membrane. The surface functions multiple and integrated as an artificial cell membrane when specific proteins and polysaccharides are coupled.

In this review, we summarize applications of nanocolloids modified by the MPC polymers for control of surface properties. There are four primary groups of applications: (i) design of nanocolloids covered with PC groups, (ii) the use of amphiphilic phospholipid polymers as nanocolloids, (iii) covering of polymeric nanoparticles with phospholipid polymers and immobilized biomolecules, and (iv) functionalization of quantum dots (QDs) with phospholipid polymer systems for cellular imaging.

Design of nanocolloids covered with PC groups

Surface modification of nanocolloids with chemically bonded or physically adsorbed PC groups is attracting increased attention. This interest is driven by fundamental studies of interfacial phenomena and also by the prospect of improving surface wettability, lubricity, and dispersion. Nanocolloid materials covered with PC groups are employed in MPC polymer grafting as well as encapsulation with phospholipids for biomedical applications. Table 1 provides a summary of surface modifications of nanocolloids with PC groups. Fig. 2 shows concept for surface modification of nanocolloids with phospholipid molecules and phospholipid polymers. Simple encapsulation of nanocolloids with phospholipid molecules and their assembly is well-known method. Using the phospholipid polymers, a solvent evaporation process for physical coating of the nanoparticles and a surface initiated-grafting from the surface of the nanocolloids have been reported. These nanocolloids covered by the PC groups have been applied in the biomedical fields. The covering of the PC groups enables the colloids to maintain the function of the colloid core within a biological environment, and enhances the dispersibility and stability of the nanocolloids in

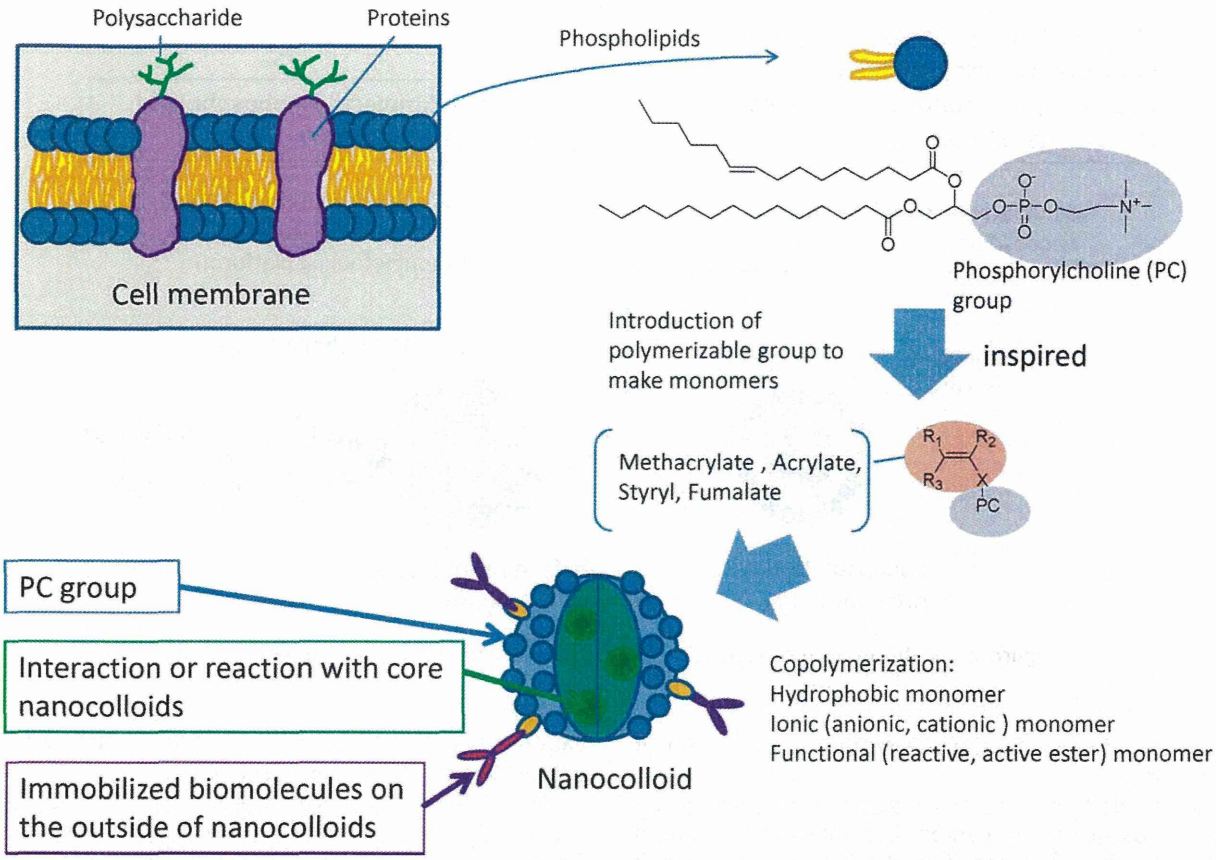

Figure 1 Functionalization of nanocolloids with phospholipid derivatives inspired from cell membrane.

Table 1 Surface modification of nanocolloid with PC group.

Core	Type of PC group	Function and biomedical potential	Ref.
SiO_2	Poly(MPC)	Nonthrombogenic materials	[35]
SiO_2	Poly(MPC)	Column Packing	[36]
SiO_2	Poly(MPC)	Analytical system	[37]
SiO_2	Trimethoxysilane derivative with PC group	Column Packing	[38]
SiO_2	DOPC	Biolabeling and Cellular Imaging	[39]
SiO_2, SnO_2	Poly(MPC) grafting	Stabilizer	[40]
SiO_2	Poly(MPC) grafting	Stabilizer	[41]
Au	Poly(MPC-*block*-DMA)	Detection system	[42]
Au	11-Mercaptoundecyl phosphorylcholine	Stabilizer	[43]
Au rod	11-Mercaptoundecyl phosphorylcholine	Cellular uptake and photothermal ablation	[44]
Ag	11-Mercaptoundecyl phosphorylcholine	Stabilizer	[45]
Ag	DSPC	Biosensing and Cell imaging tool	[46]
Fe_3O_4	Poly(MPC)	Magnetic separation	[47]
Fe_3O_4	Poly(MPC-*block*-DEA)	Imaging agent	[48]
Quantum dots	DPPC	Cell imaging tool	[49]
Carbon nanotube	Poly(MPC)	Analytical system	[50]
Carbon nanotube	POPC, BODIPY-PC	Biosensing	[51]
Polystyrene	DPPC	Lipid model	[52,53]
Polysaccharides	DPPC	Drug delivery	[54]

DOPC: 1,2-dioleoyl-sn-glycero-3-phosphocholine, DMA: 2-(*N*,*N*-dimethylamino)ethyl methacrylate, DSPC: bis(11,11′-dithiolundecyl) bis(1,1′-phosphorylcholine), DEA: 2-(*N*,*N*-diethylamino)ethyl methacrylate, DPPC: 1,2-dipalmitoyl-sn-glycero-3-phosphocholine, POPC: 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine, BODIPY-PC: 2-(4,4-difluoro-5-methyl-4-bora-3a,4a-diazas-indacene-3-dodecanoyl)-1-hexadecanoyl-sn-glycero-3-phosphocholine.

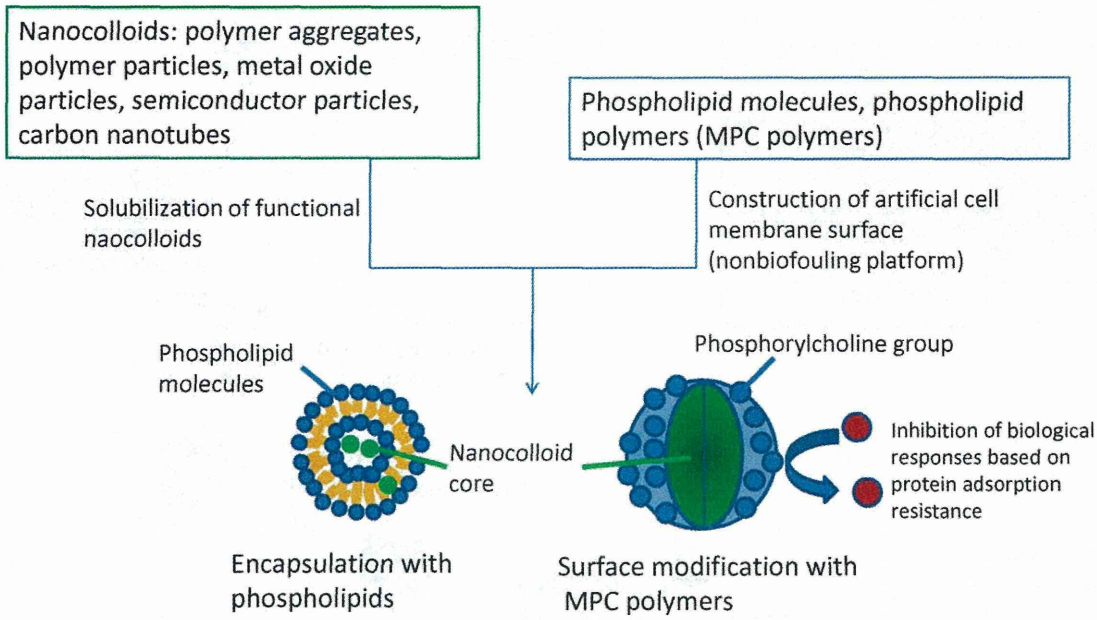

Figure 2 Hybridization of specific properties on nanocolloids by surface modification.

aqueous solution due to the prevention of adsorption of biomolecules.

Nanocolloids formed from inorganic compounds, semiconductor particles, or carbon nanotube cores have a wide range of functions and biomedical applications, which include uses as nonthrombogenic materials [35], column packing [36,38], analytical systems [37,50], cellular imaging [39,44,46,48,49], stabilizers [40,41,43,45], detection systems [42], magnetic separation systems [47], and biosensors [46,51]. Systems including nanocolloids with polymeric cores covered by PC groups have been also investigated as a lipid models [52,53] and drug delivery systems [54]. Surface modification by PC groups has been proven to be effective for biomedical applications.

Amphiphilic phospholipid polymers as nanocolloids

Although MPC polymer grafting is an effective method for providing a stable core, the method does not apply to unstable cores such as drugs, genes, and radical-sensitive materials. A simple strategy for modification of the unstable nanocolloid core involves the use of amphiphilic MPC polymer to form nanoparticles. This is induced by the presence of the MPC polymers themselves in aqueous solution. The MPC polymers may also be coated on core particles. Since amphiphilic MPC polymers with hydrophobic units form aggregations of micelles in aqueous medium via the driving force of hydrophobic interactions [55–59], the copolymers are available for solubilization of hydrophobic drugs or embedding of nanoparticles such as magnetic particles, semiconductors, and hydrophobic polymers. The amphiphilic MPC polymer with hydrophobic units and immobilization units enables integrated organization of nanobiodevices due to the dual-function provided by the core and surface. The amphiphilic MPC polymer with cationic units may be used to carry DNA vectors when DNA is condensed within the nanocolloids.

Drug delivery systems are used widely to treat diseases, especially cancerous tumors. The systems require many functions including biocompatibility, blood compatibility, hydrophilicity, and the capability to stabilize the loaded drug or DNA. Polymeric micelles consisting of amphiphilic polymers have gained increasing interest as drug carrier systems [60–62]. A representative polymer micelle formed with poly(ethylene oxide) has been used as a non-virus carrier because it is easy to control the copolymer composition, length, and shape [63]. MPC polymers are used in basic research and as drug carriers and as a shielding material for DNA vectors.

Tables 2 and 3 summarize the types of loaded drug or DNA, the MPC polymers, and immobilized biomolecules on the nanoparticles surface. Fig. 3 shows representative chemical structure of the MPC polymers. PMB, PMBN, and poly(MPC-*block*-cationic monomer) series copolymers have been mainly used as described below. Many drugs have low solubility in aqueous media. To solubilize hydrophobic drugs into aqueous media, amphiphilic MPC polymers have been investigated as drug carriers. As shown in Fig. 3, a representative copolymer is poly(MPC-co-*n*-butyl methacrylate (BMA)) (PMB) [64]. Among the PMB series with an MPC unit mole fraction of 30 mol%, the solubility of PMB against water can be controlled by the molecular weight. The water-soluble PMB30W polymer [65] (wherein ''30'' represents the unit mol% of MPC unit in PMB and ''W'' indicates solubility in water), with a molecular weight below 5×10^4 was found to be effective as a drug carrier. Surface tension measurements indicate that the surface tension of the PMB30W decreases from 10^{-2} mg/mL and becomes constant above 10^{-1} mg/mL [65]. Thus, PMB30W forms a polymeric micelle and a hydrophobic domain as a result of the formation of hydrophobic interactions of BMA in an aqueous medium. Recently, it is confirmed that the PMB can perme-

Table 2 Types of MPC polymers, loaded drug and immobilized biomolecules on the nanoparticles surface.

Polymer	Loaded drugs	Immobilized biomolecules	Diameter (nm)	Ref.
PMB	PTX	–	50	[67–69]
PLCG/PMB	SRL	–	<20	[72]
PMBN	PTX	HBs antigen	<50	[74]
PMBN	PTX	EGF	–	[75]
PMBN	PTX, FK506, CyA	IL-2	–	[76]
PMBH	Doxorubicin, PTX	Hydrazide (component of MPC polymer)	200	[77]
Poly(MPC-*block*-DPA)	Orange OT dye (not drug)	–	<30 (pH response)	[78]
Poly(FA-MPC-*block*-DPA)	Tamoxifen, PTX	Folic acid (end cap of poly(MPC) copolymer)	60–70 (pH response)	[81,82]
CMPC	ADR	–	<200	[83,84]
PIBr-Ch-g-PMPC (MPC was grafted from side chain)	PTX	–	7.5	[58]

PMB: poly(MPC-co-*n*-buthyl methacrylate(BMA)), PLCG: poly(L-lactide-co-caprolactone-co-glycolide), PMBN: poly(MPC-co-BMA-co-*p*-nitrophenylcarbonyloxyethyl methacrylate(MEONP)), PMBH: poly(MPC-co-BMA-co-methacryloyl hydrazide), FA: folic acid, DPA: *N*,*N*-diisopropylaminoethyl methacrylate, CMPC: cholessterol-end-capped poly(MPC), PIBr-Ch: poly(2-isopropyl-2-oxo-1, 3,2-dioxaphospholane-co-2-(2-oxo-1, 3, 2-dioxaphosphoroyloxyethyl-2-bromoisobutyrate-co-2-choresteryl-2-oxo-1, 3, 2-dioxaphospholane), PTX: paclitaxel, SRL: Sirolimus, CyA: cyclosporin A, ADR: adriamycin, HBs: hepatitis B surface, EGF: epidermal growth factor, IL: Interleukin.

Table 3 Types of MPC polymers, loaded DNA and immobilized biomolecules on the nanoparticles surface.

Polymer	Loaded DNA	Immobilized biomolecules	Diameter (nm)	Ref.
PMDN	sFlt-1 or GFP Plasmid DNA	HBs antigen	184 ± 54	[85]
Poly(MPC-*block*-DMA)	gWiz luc plasmid DNA	–	54.8 ± 12.9, 51.8 ± 12.2 (outer diameter of toroid, composition dependence	[86,87]
Poly(MPC-*block*-DEA)	Luciferase plasmid DNA, ODN	–	16.5×50.0 (cylinder fitting, charge ration of 1:1)	[88]
Poly(FA-MPC-*block*-DEA)	GFP Plasmid DNA	Folic acid (end cap of MPC polymer)	<200	[89]
Poly(DMAPAA-co-MPC-co-SA)	Plasmid DNA	–	280 ± 28, 290 ± 34 (before complex, composition dependence)	[90]
Poly(MPC-co-DAMA) (not target of the research)	Plasmid pCMVLacZ	–	230 ± 30 (N/P > 5)	[91]

PMDN: poly(MPC-co-*N*,*N*-dimethylaminoethyl methacrylate (DMA)-co-MEONP),DEA: *N*,*N*-diethylaminoethyl methacrylate, DMAPAA: N-[3-(dimethylamino)propyl] acrylamide, SA: stearyl acrylate, DAMA: 2-methyl-acrylic acid 2-[(2-(dimethylamino)-ethyl-methyl-amino]-ethyl ester, ODN: oligodeoxynucleotide, HBs: hepatitis B surface.

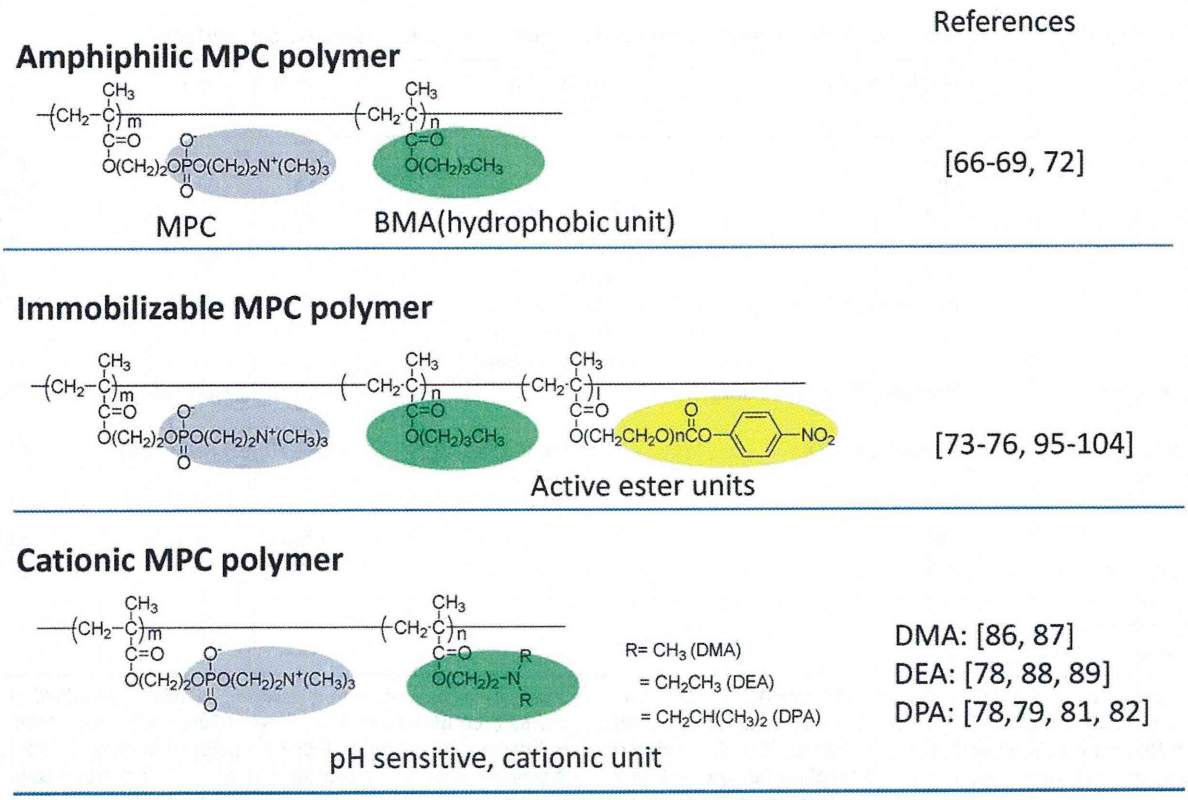

Figure 3 Chemical structure of representative phospholipid polymers, MPC polymers.

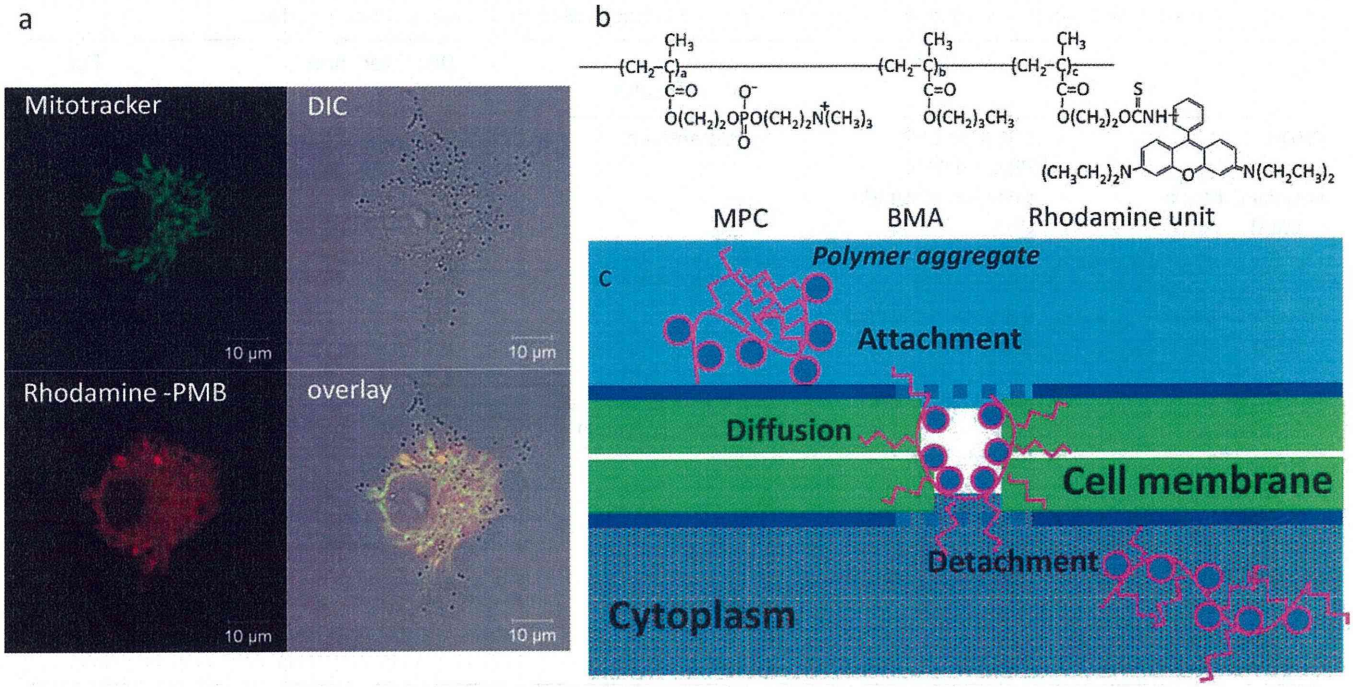

Figure 4 Direct penetration of PMB aggregates into cells by molecular diffusion mechanism [66]. (a) Confocal microscopic image after penetration of the rodamine-PMB, (b) chemical structure of rodamine-PMB, and (c) schematic representation of the diffusion mechanism through the cell membrane.

ate through the cell membrane by simple diffusion process, that is, energy-consuming endocytosis was not significantly [66]. When the PMB labeled with a specific fluorescence dye by copolymerization was introduced in cell culture medium, the inside of the cells stained within 1 min by the PMB diffusion. As the fluorescence dye, Rhodamine 6G has site-specificity to mitochondria in the cell, the Rhodamine-PMB concentrated at there (Fig. 4). This is due to flexible character of the polymer aggregate, the structure of the polymer aggregate may be changed during passing through the cell membrane.

Water-soluble and amphiphilic poly(MPC-co-BMA-co-*p*-nitrophenyloxycarbonyl poly(ethylene glycol) (MEONP)) (PMBN) were synthesized by radical polymerization [73]. An outer MEONP unit in an aqueous medium is capable of immobilizing proteins through the formation of a linkage between an active ester of MEONP and protein amino groups under mild conditions.

MPC polymers, which are responsive to pH, such as poly(MPC-*block*-2-(*N*,*N*-diethylaminoethyl methacrylate (DEA)) and poly(MPC-*block*-2-(*N*,*N*-diisopropylaminoethyl methacrylate (DPA)) were synthesized for drug delivery [78]. Poly(MPC-*block*-DPA) formed well-defined micelles at physiological pH to a greater extent than poly(MPC-*block*-DEA). Lowering of the pH of the solution was found to trigger the release of organic dye, Orange OT, due to the pKa of this component.

Polymer aggregate containing bioactive reagents

Paclitaxel (PTX), a well-known cancer drug, has poor solubility in aqueous media (<0.3 μg/mL). Konno et al. reported that the solubility of PTX could be enhanced using PMB30W (PMB30W/PTX) [67,68]. The concentration of PTX in PMB30W/PTX reached 5.0 mg/mL. The solution was transparent, and PTX did not precipitate even when the solution was stored at room temperature for 1 month. In a preliminary study, after an intravenous injection of mice with 200 mg/kg of the PMB30W aqueous solution, the mice remained calm and no weight loss was observed [67]. Soma et al. also evaluated the effect of intraperitoneal injection of PMB30W/PTX for treatment of gastric cancer. PMB30W/PTX was found to significantly reduce the numbers of metastatic nodules and to significantly reduce tumor volume relative to conventional dosages of PTX dissolved in Cremophor EL (Cremophor/PTX) [68].

Sirolimus (SRL) is a potent immunosuppressant, which inhibits the mammalian target of rapamycin, a central regulator of protein synthesis and cell growth [70]. However, SRL is an unstable agent. When degradation occurs, SRL produces an open-chain isomer, 34-hydroxy SRL, which retains less than 10% of the antiproliferative activity of the parent SRL [71]. Kim et al. reported the preparation of biodegradable polymer films for releasing nanovehicles containing SRL by combining PMB30W, poly(L-lactide-co-caprolactone-co-glycolide) (PLCG), and SRL [72]. PLCG/PMB30W/SRL films were found to reduce the production of 34-hydroxy SRL relative to PLCG/SRL films. The shielding effect provided by PMB30W nanovehicles suppresses the hydration of SRL.

Miyata et al. solubilized PTX into the hydrophobic domain of the PMBN to form a hybridized drug (PMBN/PTX) and then conjugated it with the preS1 domain of hepatitis B surface antigen for targeting IL-6 and/or immunoglobulin A binding protein [74]. Conjugation of preS1 to PMBN (preS1-PMBN) was found to strongly enhance the synergistic inhibitory effect of PTX on HepG2 cells. Shimada et al. reported the use of epidermal growth factor (EGF) conjugated to PMBN particles with PTX (EGF-PMBN/PTX) and its growth inhibitory and antitumor effects on cancer cells overexpressing EGF receptors [75]. The cytotoxicity and antitumor effects of EGF-PMBN/PTX were significantly greater than those of PMBN/PTX for EGFR-overexpressing cells, but not for an EGFR-deficient line. These results suggest that EGF-PMBN-PTX represents a more potent targeted therapy for tumors over-expressing of EGFR. Chiba et al. the showed that the proliferation of cell lines with high-affinity IL-2 receptors derived from T cell malignancies were suppressed by IL-2-conjugated (IL2-PMBN) with incorporation of PTX and cyclosporin A at lower concentrations than used conventionally [76]. IL2-PMBN-PTX was also found to inhibit the proliferation of responder cells in a human mixed lymphocyte culture at lower concentrations than the unconjugated drug.

Iwasaki et al. synthesized a water-soluble MPC polymer with hydrazide groups (PMBH) [77]. Unnatural carbohydrates with ketone groups on the cell surface of human cervical cells were obtained by treatment with levulinoyl mannosamine (ManLev) as shown in Fig. 5. The nanoparticles covered with PMB could not adhere on the cell, however, Those covered with PMBH indicated strong binding affinity to the cells. The PMBH nanoparticles immobilized with the anticancer drugs doxorubicin or PTX come into contact with either ManLev-treated or untreated cells. As a result, the anticancer drugs were selectively delivered to the ManLev-treated cell. Ligand-immobilized MPC polymers can thus be used as active targeting carriers.

A folic acid (FA)-functionalized poly(MPC-*block*-DPA) (FA-MPC-DPA) was synthesized [79]. It is well known that many malignant tissues such as ovarian, nasopharyngeal, cervical, and chorionic carcinomas consistently express high levels of folate receptors, which are accessible via the bloodstream [80]. FA has the potential to function as a tumor-targeting ligand for folate receptor. The FA-MPC-DPA micelles may be used to encapsulate PTX and tamoxifen, which are insoluble in water [81]. The loading capacity can be controlled by adjusting the composition of the block. Drug uptake studies on a colon carcinoma cell line have confirmed that FA-MPC-DPA loading of PTX and tamoxifen (approximately 100%) results in highly efficient drug uptake relative to poly(MPC-*block*-DPA). Approximately 100% of PTX and tamoxifen are loaded using FA-MPC-DPA vs 25% of tamoxifen and 40% of PTX for poly(MPC-*block*-DPA) [82]. Cholesterol-end-capped MPC polymer and poly(MPC)-grafted poly(dioxaphospholane) have also been synthesized as drug carriers [83,64,58].

Colloidal polymer complexes with DNA

Chiba et al. reported the use of cationic poly(MPC-co-*N*,*N*-dimethylaminoethyl methacrylate (DMAEMA)-co-MEONP) (PMDN) conjugated to hepatitis B surface (HBs) antigen for